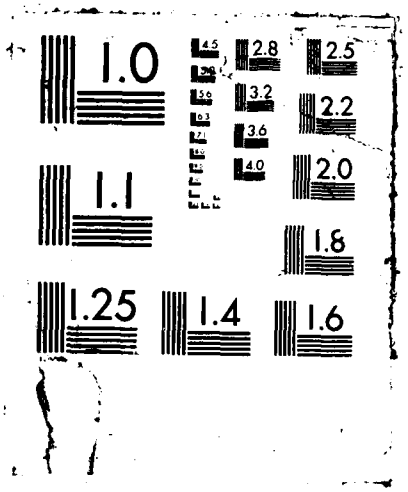
AD-A188 393 CONFIRMATION OF MULTIPLE ORGANOFLUOROPHATE HYDROLYZING ACTIVITIES IN THE..(U) CHEMICAL RESEARCH DEVELOPMENT AND ENGINEERING CENTER ABERDEEN.. M V HALEY ET AL. 1/1

UNCLASSIFIED OCT 87 CRDEC-TR-88009 F/G 6/1 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5

(4)

CHEMICAL
RESEARCH,
DEVELOPMENT &
ENGINEERING
CENTER

CRDEC-TR-88009

CONFIRMATION OF MULTIPLE ORGANOFLUOROPHATE HYDROLYZING ACTIVITIES IN THE PROTOZOAN TETRAHYMENA THERMOPHILA

by Mark V. Haley
Wayne G. Landis, Ph.D.

RESEARCH DIRECTORATE

October 1987

U.S. ARMY
ARMAMENT
MUNITIONS
CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

Distribution Statement

Approved for public release; distribution is unlimited.

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A188393

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) CRDEC-TR-88009	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION CRDEC 6b. OFFICE SYMBOL (If applicable) SMCCR-RST	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION CRDEC 8b. OFFICE SYMBOL (If applicable) SMCCR-RST	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: PROJECT NO: ETG2010 TASK NO: WORK UNIT ACCESSION NO:

11. TITLE (Include Security Classification)
Confirmation of Multiple Organofluorophate Hydrolyzing Activities in the Protozoan Tetrahymena Thermophila

12. PERSONAL AUTHOR(S)
Haley, Mark V., and Landis, Wayne G., Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM 86 Jul TO 87 Apr	1987 October	11

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Tetrahymena thermophila Organofluorophate DFPase
06	03		
08	01		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)
The use of isoelectric focusing has confirmed the existence of multiple DFPases contained within the protozoan Tetrahymena thermophila. Four distinct areas of activity were isolated, and their approximate pI's were 5.8, 5.2, 4.7, and 4.2.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
TIMOTHY E. HAMPTON	(301) 671-2914	SMCCR-SPS-T

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

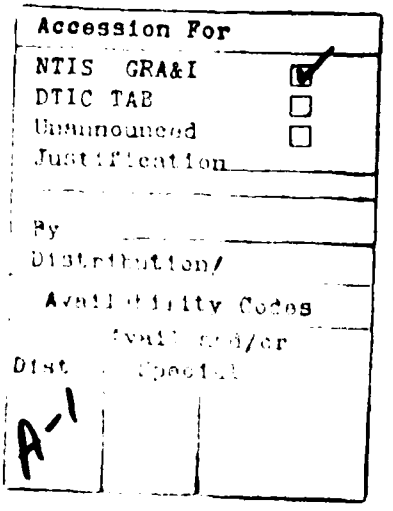
SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

PREFACE

The work described in this report was authorized under Project No. ETG2010, Aquatic Toxicology. This work was started in July 1986 and completed in April 1987.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for release to the public.



Blank

CONTENTS

LIST OF FIGURES

Blank

CONFIRMATION OF MULTIPLE ORGANOFLUOROPHATE HYDROLYZING ACTIVITIES IN THE PROTOZOAN TETRAHYMENA THERMOPHILA

1. INTRODUCTION

The term DFPase describes an enzyme capable of hydrolyzing an organofluoromonophosphate. Partially purified preparations of DFPase were subjected to flatbed thin-layer isoelectric focusing (IEF) in polyacrylamide gels with an ampholine carrier of pH 4-6.5. Until recently, the separation of DFPases was completed on molecular sizing columns (sephacryl S-300).[1] IEF provides information on the protein's isoelectric point. The isoelectric pH is the pH at which the net charge of the protein is zero.[1,2] The use of IEF has confirmed the existence of multiple DFPases contained within the protozoan Tetrahymena thermophila.

2. METHODS AND MATERIALS

2.1 Sample Preparation.

Partially purified tetrahymena extract[1] was resuspended with distilled H_2O. The material was then centrifuged for 1 hr at a relative centrifugal force of 150,000 G at 4 °C. The supernatant was passed through a Pharmacia PD-10 column filled with sephadex G-25M to reduce the salt content. The supernatant was concentrated through evaporation by using an 8-cm long Spectrapor membrane tubing with a molecular weight cut off of 6,000-8,000D. The tubing was sealed at both ends and hung in a refrigerator at 4 °C for 24 hr.

2.2 Preparation of the Polyacrylamide Gel.

Using an ampholine carrier of pH 4-6.5, flatbed, thin-layer (0.5 mm) isoelectric focusing in a polyacrylamide gel was performed according to LKB Application Note 250[4] and LKB Instruction Manual 2217.[5]

Two percent H_2PO_4 was used for the anode electrode solution and 2% ethylene diamine was used for the cathode electrode solution. Isoelectric focusing was run at 2,000 v 20 ma and 10 w using an LKB 2197 power supply. The concentrated tetrahymena samples were held in place on the gel with 5 x 10 mm filter pads. The maximum sample volume placed on the pads was 30 µl. Three markers were used to determine the pH gradient. The samples were run for 30 min. The pads were removed and the samples were run for an additional 4.5 hr.

After the run was completed, a sample path was removed from the gel and cut into 22 sections 0.5 cm wide. Proteins were eluded from the gel sections by placing them into 0.5 ml of Hoskin's buffer[6] at 4 °C for 24 hr. The remaining portion of the gel was stained in a 0.5% solution of Coomassie blue R-250; the stained protein bands were analyzed on a laser densitometer.

2.3 DFP Assays.

The eluted proteins were assayed for activity according to methodology described earlier.[6] Slight modifications were made by using 6 x -3M DFP solutions. The Orion 901 meter was calibrated to 1 and 50 µm of NaF.

3. RESULTS

Sixty-six protein bands were resolved by the laser densitometer. Four distinct areas of activity were isolated at slice numbers 8, 12, 15, and 18 (Figure 1). Slice 8 had a small amount of protein and could be an artifact. However, slices 12 and 15 had two bands of protein and slice 18 had three. (Figures 2 and 3). The approximate isoelectric points for slices 8, 12, 15, and 18 were 5.8, 5.2, 4.7, and 4.2 respectively (Figure 4). Slice 18 had the greatest amount of activity with slices 12 and 15 being approximately equal.

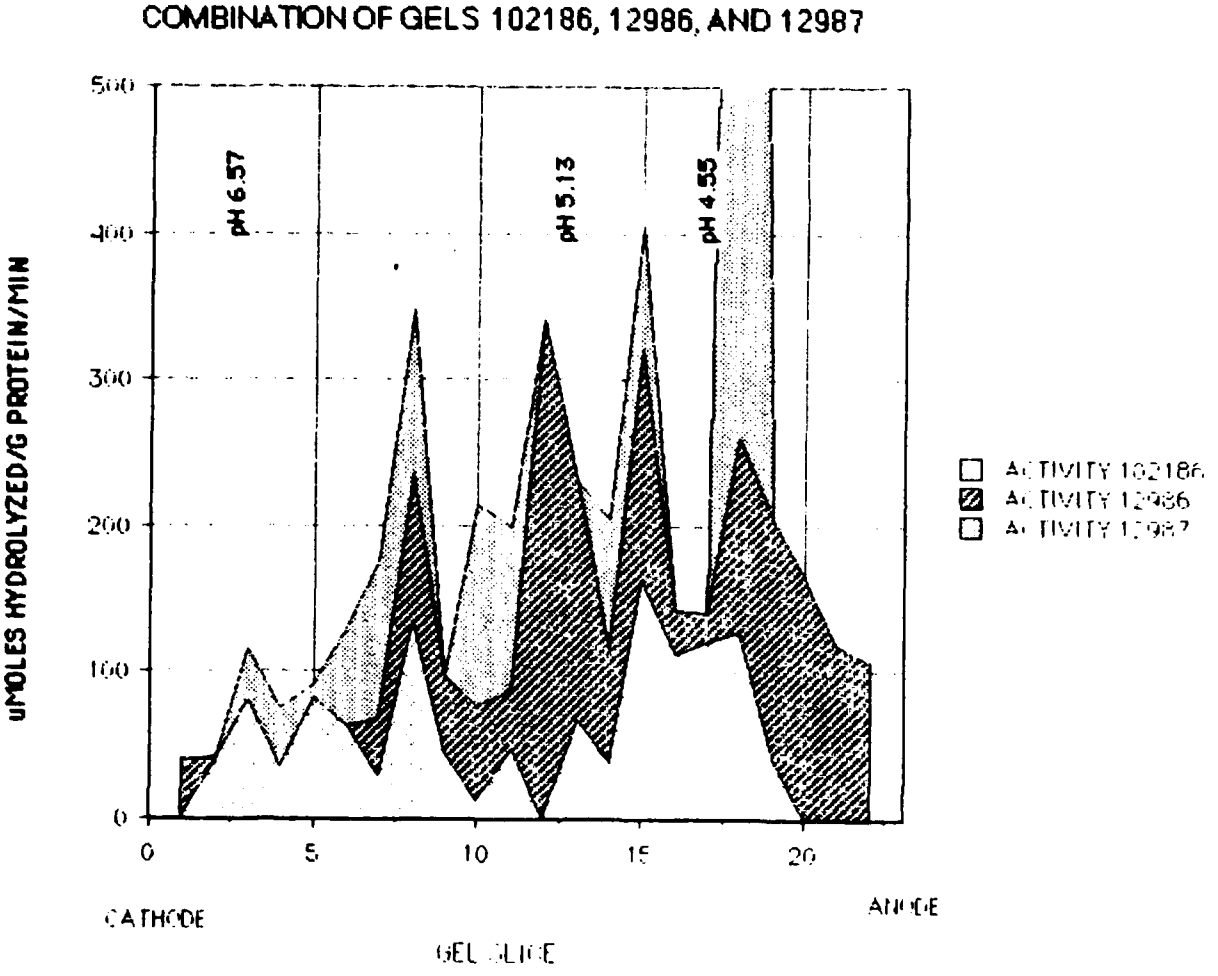


Figure 1. DFPase Activities of Partially Purified Preparations of Tetrahymena thermophila. The area graph combines the activities of the three runs to make the peaks of activity easier to see.

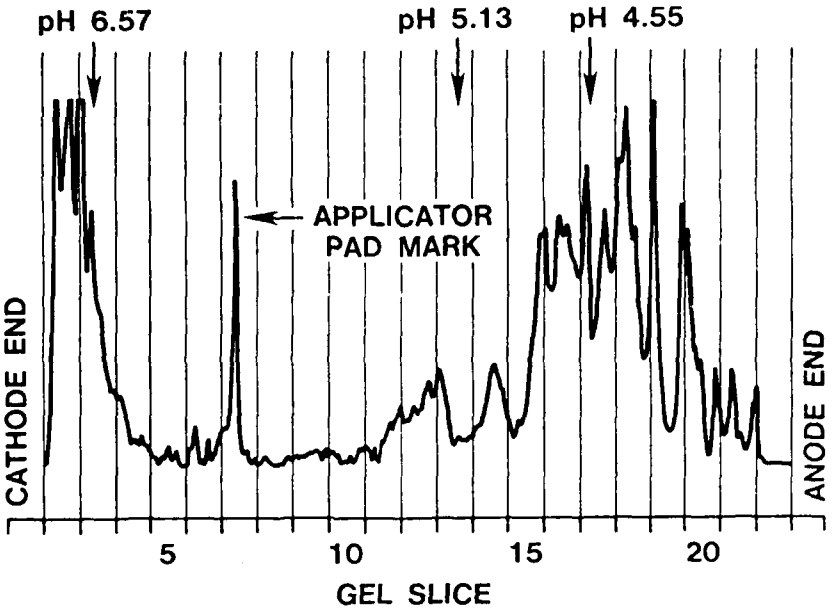


Figure 2. Laser Densitometer Scan of a Stained Gel After Tetrahymen Samples Were Run for 5 Hr

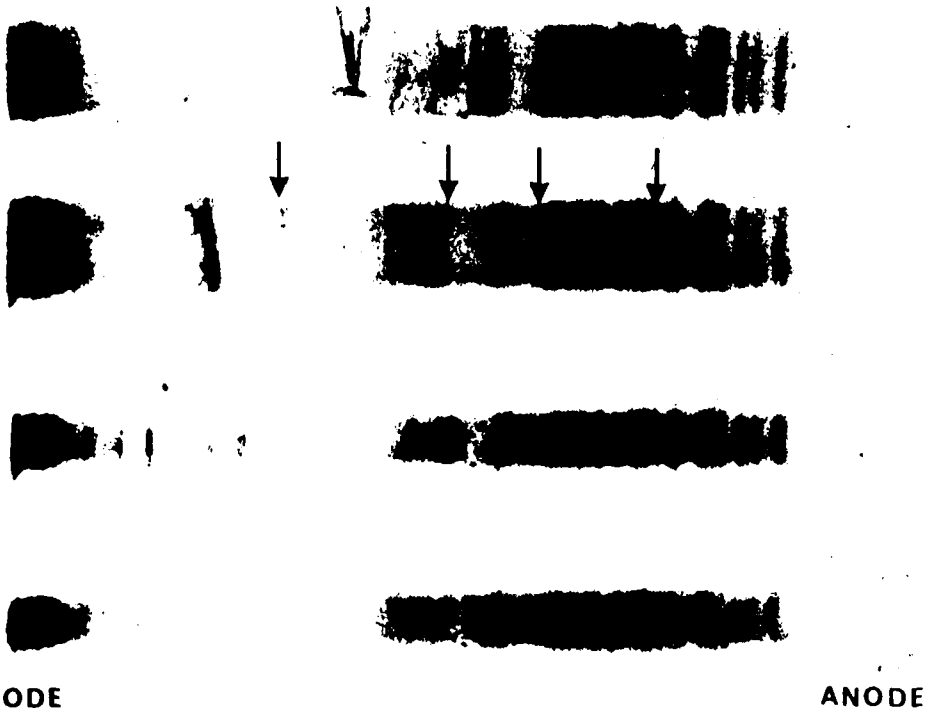


Figure 3. An Isoelectricfocusing Gel of Partially Purified Preparations of Tetrahymena Stained With a Solution of 0.5% Coomassie Blue R-250. The arrows indicate areas of high activity.

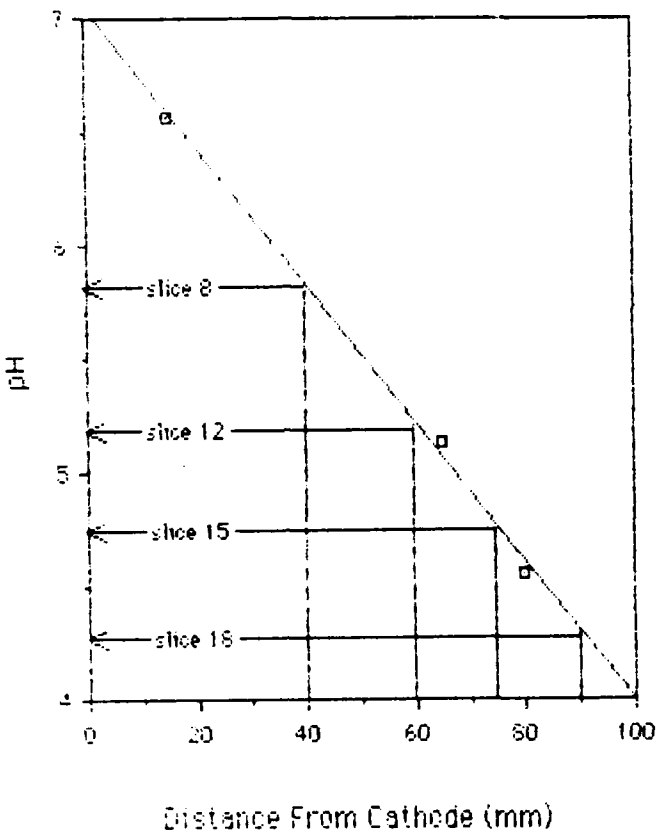


Figure 4. Beer-Lambert Plot of Isoelectric Markers and the Computation of Gel Slice Isoelectric Points

4. DISCUSSION

These experiments have confirmed the multiple DFPase entities as first discovered by sephacryl molecular sizing columns. Four areas of activity have been isolated (Figures 1 and 2). The activity in gel slice 8 may be an artifact. The amount of protein eluded from slice 8 was to small for reliable activity calculations. Slice 12, 15, and 18 were the major areas of activity.

The next stage of research is to run the partially purified preparations of DFPase on a preparative flatbed electrofocusing in a granulated gel. This method would allow applying a maximum sample size of 4.5 ml. These gels will separate such a large volume that the eluded protein bands would have a concentration large enough to be applied to molecular sizing columns.

LITERATURE CITED

1. Landis, W.G., Haley, M.V., Johnson, D.W., Durst, H.D., and Savage, R.E., Jr. "Discovery of Multiple Organofluorophosphate Hydrolyzing Activities in the Protozoan Tetrahymena thermophilla". J. of Applied Toxicology, Vol. 7, p 35-41. (1987).

2. Righetti, P.G., Isoelectric Focusing: Theory, Methodology and Applications, Works, T.S., and Burdon, R.H. eds, Elsevier Biomedical Press, New York, p 7, (1984).

3. Suelter, C.H., A Practical Guide to Enzymology, John Wiley and Sons, Inc., New York, New York, pp 139-141, (1985).

4. Winter, A., Ek, K., and Andersson, U.B., Analytical Electrofocusing in Thin Layers of Polyacrylamide Gels, Application Note 250, LKB, Gaithersburg, MD, 20877, (1977).

5. LKB, Ultromould - For Casting Polyacrylamide Gels in Thickness Between 0.1 and 0.5 mm for Electrofocusing With Ampholine Carrier Ampholytes, Instruction Note 2217, LKB, Gaithersburg, MD, 20877,

6. Landis, W.G., Haley, M.V., and Johnson, D.W., "Kinetics of the DFPase Activity in Tetrahymena thermophila," J. of Protozoology, Vol. 2, p 33, (1986).

END

DATE

FILMED

MARCH

1988

DTIC